REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 00537-00900K.

Respectfully submitted,

Date: May 30, 2002

Jeffrey D. Hsi
Reg. No. 40,024

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 1, line 26, has been amended as follows:

The [amehibian] amphibian peptide bombesin, pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (SEQ ID NO:1) (Anastasi et al., Experientia 27: 166-167 (1971)), is closely related to the mammalian gastrin-releasing peptides (GRP), e.g., the porcine GRP, H_2N-Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-(NH_2) (SEQ ID NO:2) (McDonald et al., Biochem. Biophys. Res. Commun. 90: 227-233 (1979)) and human GRP, H_2N-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met (NH_2) (SEQ ID NO:3). Bombesin has been found to be a growth factor for a number of human cancer cell lines, including small-cell lung carcinoma (SCLC), and has been detected in human breast and prostate cancer (Haveman et al., eds. Recent Results in Cancer Research - Peptide Hormones in Lung Cancer, Springer-Verlag, New York: 1986). A number of these cancers are known to secrete peptide hormones related to GRP or bombesin. Consequently, antagonists to bombesin have been proposed as agents for the treatment of these cancers.

Paragraph beginning at page 13, line 3, has been amended as follows:

Examples of preferred bombesin or GRP peptides are:

D-β-Nal-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Phe-NH_2,

D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ethylamide,

p-Glu-Gln-Trp-Ala-Val-Gly-His-statine-amide (SEQ ID NO:4),

D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]-D-Phe-NH_2,

D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2,

D-Cpa-Gln-Trp-Ala-Val-D-Ala-His-β-Leu-NH_2,

D-Cpa-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]-Phe-NH_2.

Paragraph beginning at page 13, line 22, has been amended as follows:

An example of a preferred GRF peptide of the invention is Tyr-Ala^2-Asp-Ala-Ile-Phe-Thr-Asn-SerΨ[CH_2NH]Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH_2 (SEQ ID NO:5); most preferably, the peptide contains, D-Ala, N-methyl-D-Ala, or [a] alpha-aminobutyric acid in position 2. (Non-peptide bonds in which the peptide bond is reduced are symbolized herein by "Ψ[CH_2NH]" or "Ψ".)

Paragraph beginning at page 15, line 9, has been amended as follows:

Fig. 2 is a series of amino acid sequences of naturally occurring peptides of which peptides of the invention are analogs (SEQ ID NOs:13-16, respectively).

Paragraph beginning at page 15, line 12, has been amended as follows:

[Fig. 3 is] Figs. 3A ad 3B are a series of amino acid sequences of naturally occurring peptides of the VIP peptide family, of which GRF peptides of the invention are analogs (SEQ ID NOs:17-26, respectively).

Paragraph beginning at page 15, line 18, has been amended as follows:

Fig. 6 is a graph showing the antagonism of GRF stimulated GH secretion by Ser$^9\Psi$[CH_2NH]Tyr^{10} GRF(1-29)NH_2 (SEQ ID NO:5). We now describe the structure, synthesis, and use of the preferred embodiments of the invention.

Paragraph beginning at page 16, line 17, has been amended as follows:

The synthesis of the bombesin antagonist pGlu-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:7) follows. Other bombesin, GRP, or GRF antagonists can be prepared by making appropriate modifications of the following synthetic methods.

Paragraph beginning at page 16, line 22, has been amended as follows:

The first step is the preparation of the intermediate pGlu-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbonyl)-LeuΨ[CH_2NH]Leu-benzhydrylamine resin, as follows.

Paragraph beginning at page 18, line 14, has been amended as follows:

The product is found to be homogeneous by HPLC and TLC. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide. The presence of the LeuΨ[CH_2-NH]Leu bond is demonstrated by fast atom bombardment mass spectrometry. pGlu-Gln-Trp-Ala-Val-Gly-His-PheΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:6) and pGlu-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:7) or other peptides are prepared in similar yields in an analogous fashion by appropriately modifying the above procedure.

Paragraph beginning at page 24, line 21, has been amended as follows:

Solid-phase synthesis of the peptide BIM-26120, pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH_2 (SEQ ID NO:4), was accomplished through the use of the following procedures in which alpha-t-butoxycarbonyl statine (prepared by the procedure of Rich et al., J. Org. Chem. 1978, 43, 3624) is first coupled to methylbenzhydrylamine-polystyrene resin. After acetylation, the intermediate p-Glu-Gln-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbonyl)-Sta-methylbenzhydrylamine (SEQ ID NO:9) resin is prepared. The synthetic procedure used for this preparation follows in detail:

2. Incorporation of alpha-t-butoxycarbonyl statine on methylbenzhydrylamine resin.

Paragraph beginning at page 30, line 27, has been amended as follows:

Solid phase synthesis of $Ser^{9}\Psi$[CH_2NH]Tyr^{10}GRF(1-29), Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn- SerΨ[CH_2NH]Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH_2 (SEQ ID NO:5), was carried out as follows.

Paragraph beginning at page 31, line 16, has been amended as follows:

The resin bound peptide was elongated by repeating cycles (a-j) to give Boc-Tyr-Arg-Lys-Ala-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-methylbenzhydrylamine (SEQ ID NO:10). The Boc group is then removed by TFA treatment. Boc-serine aldehyde (0.75 mmoles), prepared by the method of Fehrentz and Castro (1), is dissolved in 5 ml of dry DMF and added to the resin TFA salt suspension followed by the addition of 100 mg (2 mmoles) of sodium cyanoborohydride (2, 3). After stirring for 1 h, the resin mixture is found to be negative to ninhydrin reaction (1 min) indicating complete derivatization of the free amino group.

Paragraph beginning at page 40, line 14, has been amended as follows:

Growth of NCI-H69 xenografts and the tumor growth inhibitory activity of the bombesin antagonist BIM-26100 (pGlu-Gln-Trp-Ala-Val-Gly-His-PheΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:6)) are illustrated as tumor growth curves in Fig. 1, and relative tumor sizes in Table 2. Administration of BIM-26100 as a s.c. infusion around the tumor significantly inhibited tumor growth. The effectiveness of the antitumor activity of BIM-26100 is evident in view of the large inoculum of NCI-H69 tumor cells (i.e., the equivalent of 5 confluent 75 cm^2 cell culture flasks per animal) and the agglomerated condition of the cells. In confluent flasks, NCI-H69 agglomerates are macroscopically visible and together resemble a metastatic tumor colony. Many such tumor colonies were implanted per animal. The dose of BIM-26100 was arbitrarily selected on the bases of compound availability and is not optimal. Higher doses of BIM-26100 may be administered, as indicated by body weight gain (minus tumor weight) gain during the course of treatment (Table 3). This suggest BIM-26100 completely lacks local or systemic toxicity and is useful therapeutically as an anti-growth factor with anti-tumor effects.

Paragraph beginning at page 41, line 13, has been amended as follows:

The purified analogs were assayed in a 4-day primary culture of male rat anterior pituitary cells for growth hormone (GH) release, as described by Hocart et al. (1988, supra) and

Murphy and Coy (1988, Peptide Research 1:36). Potential antagonists were retested in the presence of GRF(1-29)NH_2 (SEQ ID NO:11) (1 nM). The results are shown in Figs. 4-6, in which different dosages of the analogs were measured against GRF.

Paragraph beginning at page 41, line 21, has been amended as follows:

The incorporation of the reduced peptide bond isostere in the N-terminal region of GRF(1-29)NH_2 (SEQ ID NO:11) produced very weak agonists and one antagonist with an IC_{50} of approximately 10μM.

Paragraph beginning at page 41, line 25, has been amended as follows:

Placement of the pseudopeptide bond between the N-terminal 9^{th} and 10^{th} residues produced the analogue [$Ser^9\Psi[CH_2NH]Tyr^{10}$]-GRF(1-29)$NH_2$ (SEQ ID NO:5) (peptide VIII). This analog was found to be inactive in the potency assay, and was therefore tested for antagonist activity in the presence of a stimulating dose of GRF(1-29)NH_2 (SEQ ID NO:11) (1 nM). The results are shown graphically in Fig. 6. [$Ser^9\Psi[CH_2NH]Tyr^{10}$]-GRF(1-29)$NH_2$ (SEQ ID NO:5) was found to be an antagonist in the 10μM range vs 1 nM GRF. Earlier conventional structure-activity studies with the same peptide had elucidated a more potent antagonist, namely [N-Ac-Tyr^1, D-Arg^2]GRF(1-29)NH_2 (Robberecht et al., J. Endocrinology, 1985, 117, 1759). This analog had an IC_{50} of approximately 1μM in an assay for adenylate cyclase activity in rat anterior pituitary homogenates.

The table beginning at page 45, line 1, has been amended as follows:

Table 1

Code	Structure	3T3 GRP Receptor IC50(nM)	Thym. Uptake IC50(nM)
BIM-26092	Gly-Asn-His-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:12) Neuromedin C	242	466
BIM-26095	pGlu-Gln-Trp-Ala-Val-D-Ala-His-LeuΨ[CH_2NH]Leu-NH_2 Litorin	2623	1209
BIM-26100	pGlu-Gln-Trp-Ala-Val-Gly-His-PheΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:6) Litorin	23	26
BIM-26101	pGlu-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 (SEQ ID NO:7) Litorin	118	296
BIM-26105	D-Ala-Asn-His-Trp-Ala-Val-D-[ALa] Ala-His-LeuΨ[CH_2CH]Leu-NH_2 Neuromedin C	107	107
BIM-26106	desGly-D-Ala-His-Trp-Ala-Val-D-[ALa] Ala-His-LeuΨ[CH_2CH]Leu-NH_2 Neuromedin C	401	354
BIM-26107	D-Phe-His-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 Neuromedin C	199	154
BIM-26108	N-Ac-D-Ala-His-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 GRP (19-27)	841	>1000
BIM-26113	D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 Litorin	5.8	9

BIM-26114	D-Nal-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Leu-NH_2 Litorin	23.5	28
BIM-26120	pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH_2 (SEQ ID NO:4) Litorin	150	165
BIM-26122	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 Litorin	5.9	28.6
BIM-26136	D-Nal-Gln-Trp-Ala-Val-Gly-His-LeuΨ[CH_2NH]Phe-NH_2 Litorin	1.4	3.3
BIM-26182	D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2 Litorin	0.88	4.77

In the claims:

Claim 4 has been amended as follows:

4. (Amended) The therapeutic peptide of claim 2 of the formula:

p-Glu-Gln-Trp-Ala-Val-Gly-His-statine-amide (SEQ ID NO:4).